FEBS Letters 589 (2015) 2477–2486

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

Elastin-like polypeptides as models of intrinsically disordered proteins

Stefan Roberts [1], Michael Dzuricky [*,1], Ashutosh Chilkoti

Duke University, Durham, NC, United States

ARTICLE INFO

Article history:
Received 13 July 2015
Revised 18 August 2015
Accepted 19 August 2015
Available online 29 August 2015

Edited by Wilhelm Just

Keywords:
Elastin-like polypeptide
Intrinsically disordered protein
Phase transition
Biopolymer
Tandem repeat
Protein engineering

ABSTRACT

Elastin-like polypeptides (ELPs) are a class of stimuli-responsive biopolymers inspired by the intrinsically disordered domains of tropoelastin that are composed of repeats of the VPGXG pentapeptide motif, where X is a "guest residue". They undergo a reversible, thermally triggered lower critical solution temperature (LCST) phase transition, which has been utilized for a variety of applications including protein purification, affinity capture, immunoassays, and drug delivery. ELPs have been extensively studied as protein polymers and as biomaterials, but their relationship to other disordered proteins has heretofore not been established. The biophysical properties of ELPs that lend them their unique material behavior are similar to the properties of many intrinsically disordered proteins (IDP). Their low sequence complexity, phase behavior, and elastic properties make them an interesting "minimal" artificial IDP, and the study of ELPs can hence provide insights into the behavior of other more complex IDPs. Motivated by this emerging realization of the similarities between ELPs and IDPs, this review discusses the biophysical properties of ELPs, their biomedical utility, and their relationship to other disordered polypeptide sequences.

Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

1. Introduction

Elastin is an extracellular matrix protein critical for the elastic properties of extensible tissues such as ligaments and blood vessels [1,2]. It is composed of a matrix of cross-linked tropoelastin, a 72kDa protein containing alternating hydrophobic and crosslinking domains [3,4]. The mechanical properties of elastin are a consequence of its low sequence complexity hydrophobic domains that are composed of 80% valine, proline, glycine, and alanine [3,4]. These domains are highly disordered under native conditions, and this structural variability allows for high degrees of mechanical elastic recoil [5,6]. Elastin was one of the earliest studied examples of an intrinsically disordered protein because its biological functionality was assumed to be exclusively mechanical, and therefore its inherent disorder did not violate the prevailing dogma of "structure dictates function" in proteins [4].

Elastin-like polypeptides (ELPs) are a class of artificial peptide polymers composed of a VPGXG pentapeptide repeat unit—where X can be any amino acid except proline. This repeat unit is derived from the hydrophobic domain of tropoelastin [7,8]. These recombinant polymers display lower critical solution temperature (LCST) phase behavior that leads to the formation of an insoluble coacervate phase above the cloud point of the polymer, similar to tropoelastin [3]. The LCST of ELPs can be tuned to respond to different stimuli such as temperature [8,9], the type and concentration of salts [10], other cosolutes such as proteins [11,12], pH [13,14], and light [15]. Their reversible phase behavior has led to their stimuli-triggered self-assembly into nanoparticles [16–18] and hydrogels [17,19,20] that are finding application in drug delivery [9,21–24] and tissue engineering [25,26].

The explosion of research on intrinsically disordered proteins (IDPs) and intrinsically disordered regions (IDRs) in the last decade has led to a paradigm shift in understanding the structure–function relationship of proteins [27,28]. Disorder is, in fact, not limited to only mechanically active proteins, but also plays a crucial role in a host of other cellular functions [29,30]. In an attempt to understand the origins of disorder in these proteins and how disordered proteins may adopt one or multiple structures in response to a biological signals, researchers have looked to polymer physics studies that were largely carried out on synthetic disordered polymers [31,32]. Protein polymers such as ELPs occupy a niche that is midway between synthetic polymers and IDPs. IDPs owe their disorder to repetitive, low complexity sequences of limited hydrophobicity [28], and ELPs are an extreme example of this amino acid syntax (Fig. 1), as they are completely disordered polypeptides composed only of repeats of short peptide motifs. Hence, we suggest they can be thought of as representing a minimal, prototypical IDP. Herein, we analyze the relationship of ELPs to IDPs and seek to explore

* Corresponding author.
E-mail address: mjd46@duke.edu (S. Roberts).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.febslet.2015.08.029
0014-5793/Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.

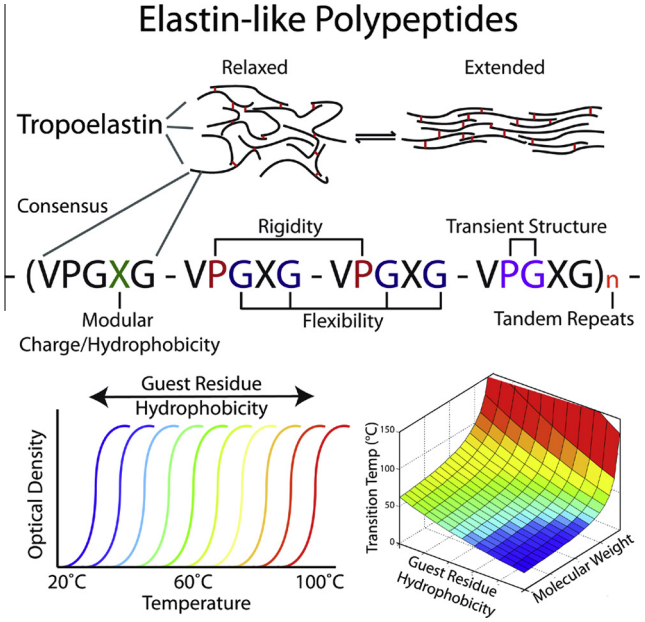

Fig. 1. Elastin-like-polypeptides (ELPs): Disorder encoded at the sequence level. ELPs are tandem repeat proteins derived from tropoelastin. The consensus repeat unit VPGXG promotes high conformational flexibility at low temperatures and a disordered molten globule aggregate at higher temperatures. Closer analysis of these engineered tropoelastins shows a variety of parameters that control the chain's disordered state, namely the proline/glycine content, number of tandem (n) repeats, and guest residue composition (X). Careful consideration of these parameters in the chain design provides exquisite control over the aforementioned temperature of transition. Perhaps more remarkably, this transition is completely reversible. Due to their extreme low complexity and moderate hydrophobicity, ELPs can serve as a fundamental IDP and further study into their syntax will provide crucial insight into other elastomeric or intrinsically disordered proteins.

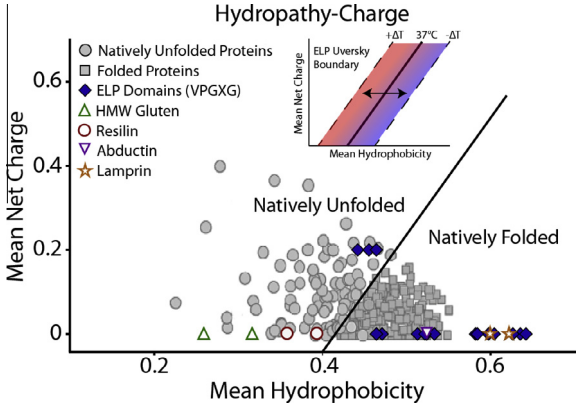

Fig. 2. Tandem repeat proteins plotted as a function of charge vs. hydrophobicity. The Uversky plot is the archetypical method for determining protein disorder. It plots the normalized overall net charge of a protein chain against its average hydrophobicity. The line divides proteins that are natively unfolded, what we have termed intrinsically disordered, and proteins that fold into a stable conformation in space. *Here we are plotting canonical ELP sequences and other elastomeric repeat proteins with experimentally confirmed large degrees of disorder with data (gray points) from Uversky's original study* [34]. Immediately evident is ELP's ability to span both the natively folded and unfolded regions of the Uversky plot via modulation of the guest residue. This characterization could in part be explained by ELPs temperature dependent aggregation which could be considered a type of ordered conformation. ELP aggregation is dependent on temperature and repeat length, both of which the Uversky plot cannot take into account. The figure inset shows how the divide between ordered and disordered domains, determined at physiological temperatures, may shift for elastomeric proteins like ELPs. Note that for disordered proteins which display upper critical solution temperature (UCST) phase separation, such as resilin and abductin, which also span the divide, the boundary would shift in the opposite direction with temperature. Although the hydropathy-charge ratios can give some indication of the propensity of a protein to fold, clearly other factors must contribute to chain conformations sampled by a polypeptide chain.

how cross-talk between the fields of recombinant peptide polymers and IDPs can help push both fields forward.

2. Disorder at the sequence level

2.1. Hydropathy-charge

There has been significant effort in the past few decades to understand the role that primary sequence plays in determining the tertiary structure of proteins. As the field of disordered proteins has expanded, it has also become clear that disorder, in addition to structure, may be encoded at the amino acid level [28]. This realization has led researchers to pursue an algorithmic approach to parsing protein databases for sequences indicative of disorder and has led to the determination of several key identifiers of disorder in a protein sequence [27,33]. One of the better recognized descriptions of crucial determinants in protein disorder stems from Uversky et al.'s analysis of the impact of charge and amino acid hydropathy on the tendency of a particular sequence to form a compact globular structure [34]. This analysis uses a normalized plot of hydrophobicity and net charge to determine whether a sequence has the properties to preclude the formation of a folded structure.

Analyzing the canonical ELP sequence $(VPGVG)_n$ by this algorithm places it firmly in the region of compact, folded proteins with a zero net charge and a moderately high hydrophobicity (Fig. 2). This finding is inconsistent with the prevailing notion of ELPs as highly disordered proteins but can be readily resolved by considering that Uversky's algorithm is based on protein conformations at physiological temperatures as $(VPGVG)_n$ is expected to be collapsed, or "folded", at this temperature [9]. Altering the guest residue of the more general ELP, $(VPGXG)_n$, allows precise mobility along both axes of Uversky's plot, hence allowing precise control of the propensity for disorder. ELPs with highly hydrophilic or charged residues can even have a transition temperatures high enough that they are inaccessible in aqueous solutions [11], exhibiting the thermal stability often associated with and used to test for new disordered proteins [27]. The ability to alter the degree of disorder, or propensity to collapse, in an ELP may be useful for researchers looking to understand the effects that particular amino acids have in driving the stability of aggregates in a biological system. It also suggests that there may be coil-globule transitions for many biologically active IDPs, a property already observed in a number of other elastomeric proteins shown in Fig. 2 [35,36].

2.2. Tandem repeats

Another common aspect that many IDPs and IDRs share with ELPs is the frequency of low sequence complexity tandem repeats, defined as patterns of repeats of highly similar amino acids [28,37]. The presence of low sequence complexity regions is unsurprising given the inherent bias toward disorder promoting amino acids. These regions in IDPs can be composed of simple repeats such as polyserine or polyglutamine or more complex repeats such as those found in resilin [38]. An analysis of the Protein Data Bank (PDB) by Jorda et al. revealed that greater degrees of disorder in a protein sequence are associated with more perfect tandem repeats, or those with more precise spacing of the same amino acids across repeats [39]. In a similar vein, Das and Pappu recently simulated the aggregation propensities of a library of polyampholytic IDPs and found that, for these highly zwitterionic sequences, more perfect degrees of amino acid repetition increased the propensity to disorder compared to more irregularly spaced repeats [40].

ELPs offer some interesting insights into the effects of tandem repeats on disorder, as they are protein polymers that can be designed to have a perfect repeat structure (Fig. 1). The guest residue of canonical ELP sequences is not limited to the inclusion of a single amino acid throughout the polymer chain and mixing different amino acids is a common way of precisely controlling their transition temperature [9,41]. Comparing "well-mixed" ELPs to recombinant block copolymer ELPs with the sequence $(VPGX_1G)_n$–$(VPGX_2G)_m$ demonstrates how important this tandem repeat mixing parameter can be. Block copolymers that are sufficiently amphiphilic—created, in this example, by choosing the guest residue X_1 to be significantly more hydrophobic than X_2—will self-assemble into micelles above critical micellization temperature (with $(VPGX_1G)_n$ in the core), whereas the well-mixed guest residues of $(VPG[X1/X2]G)_n$ will maintain solubility through the entire polymer chain [9,17,41].

2.3. Disordered, but not random

The precise nature of the structure–function relationship of elastin was a point of contention for many years. Initial theories proposed by Flory argued that elastin was a random-conformation network whose elastic properties were a result of the loss of entropy upon polymer stretching [42]. Gosline proposed a similar random-coil theory where the entropic driving force necessary for elastin's elasticity is derived from an increase in solvent entropy caused by hydrophobic interactions along the backbone of ELP [43,44]. Urry later postulated that elastin and ELPs were not random coil but that, they were composed of repeat units of PG beta turns that exhibit an extended, and ordered, beta spiral conformation [7,45]. Work by Tamburro also suggests the presence of type II β turns; however his model predicts non-recurring, dynamic beta turns which interconvert between disordered and hydrogen bonded states [46,47].

Early work in this field was plagued by many of the same issues now facing the field of IDPs. Unable to use X-ray crystallography to generate high-resolution protein structures for elastin, more indirect methods had to be used to evaluate ELPs. Comparison of solid-state NMR chemical shift values to those expected for β-sheet, α-helix, or random coil, for example, suggested a system devoid of secondary structure [48–51]. It should be noted that there is a significant lack of solution NMR analysis of elastin or ELPs, presumably due to relative insolubility of native elastin and the poor peak dispersion at high concentrations and typical NMR working temperatures, though one such analysis of a triblock elastin mimic was undertaken by Wright el al. [52]. However, circular dichroism (CD) spectra of both native elastin and ELPs show distinct deviation from a completely random coil network and suggest their propensity to form the beta turns and PPII structure proposed by Urry [53–55].

To resolve confusion surrounding the true structure of ELPs, some researchers have carried out molecular dynamics (MD) simulations, which can provide atomistic resolution of ELP chain conformation in solution. While early MD simulations were restricted to short time scales or short sequences because of computational limitations, advances in computing power have made simulations a useful functional tool in the analysis of disorder. Results from simulations from Darwin et al. [35], Rauscher et al. [56], and Yingling and coworkers suggest that ELPs are predominantly random coil polymers in which each repeat is independently capable of transiently sampling beta turn and polyproline type II (PPII) structure [57]. Aggregation is driven by small conformational changes in the polymer followed by abrupt changes in backbone solvation and the system entropy [57]. It should be noted that the correctness of the term "random coil" has been questioned for a number of years. Since proteins and protein-based polymers are not subject to true Gaussian conformations, the term "statistical coil" may be more accurate [58–60]. Understanding the distinction, we nevertheless refer to the disordered portion of proteins in this review as "random coil" to maintain consistency with cited literature for both ELPs and IDPs.

The ability for ELPs to sample transient structured conformations is an important characteristic that they share with many other IDPs. ELPs exist on an "egg carton" like energy landscape capable of sampling a number of conformations until a thermodynamic driving force induces a change. This property is not unlike that exhibited by IDPs which have evolved to bind multiple different partners or to undergo a structural shift upon binding [28]. For most IDPs, the thermodynamic driving force that triggers their conformation change is interaction with another partner, whereas the trigger in ELPs is a change in temperature, but the concept of sampling structural conformations until a trigger drives an IDP or ELP to adopt a new conformation or set of conformations is similar.

3. Aggregation and phase behavior

3.1. Disorder in aggregation

ELPs owe their highly disordered nature to systematic contributions from proline and glycine to solvation of the polypeptide backbone (Fig. 1). These two amino acids are both necessary for the intrinsic disordered nature of ELPs, but for opposing reasons. The lack of a true side chain for glycine allows it an extremely high degree of chain mobility, permitting the protein to sample a variety of chain conformations. This property allows it to contribute to structured or unstructured domains, but does not induce the formation of either [61]. Proline, on the other hand, promotes rigidity on all length scales of the ELP, prohibiting the formation of stable secondary structure [61]. These combined contributions from proline and glycine work together to keep ELPs at varying degrees of disorder in both the solvated and aggregated state [37]. Even in an aggregated state, ELPs retain a high degree of water, allowing chains to continually interpenetrate one another while the rigidity derived from proline prevents the formation of hydrogen bonds that can drive the formation of a stable secondary structure.

The concept of disorder in a bound, or aggregated state, runs counter to the logic of conventional structure–function relationships in globular proteins but is somewhat common among IDPs [28,62]. Tompa and Fuxreiter have coined the term "fuzziness" to describe conformational disorder in protein complexes [62], and have reviewed its prevalence in IDPs extensively [37,63]. Fuzziness in binding adds adaptability and reversibility to protein interactions, thereby assisting their regulation of sensitive biological feedback loops. Their prevalence in the regulation of transcription and translation has been most extensively studied; however, IDPs displaying conformational disorder in a bound state can range from the static amyloid states of prions to the complete dynamic disorder of the T-cell receptor ζ chain [62,64].

3.2. Amyloid formation

Implicit to the observation that proline and glycine content contributes to the dynamic disorder of ELP in a transitioned state is the notion that alterations to these residues will result in more stable aggregates. To address the impact of proline and glycine on aggregated states, Rauscher et al. systematically analyzed the propensity of a variety of elastomeric proteins to aggregate, including elastin [61]. Similar to Uversky's observations that there is a critical threshold of charge and hydropathy for a protein to be disordered in solution, Rauscher has shown that there is an approximate threshold of proline and glycine content that controls the propensity for elastomeric proteins to form amyloids (Fig. 3), an irreversible aggregation event distinct from reversible phase

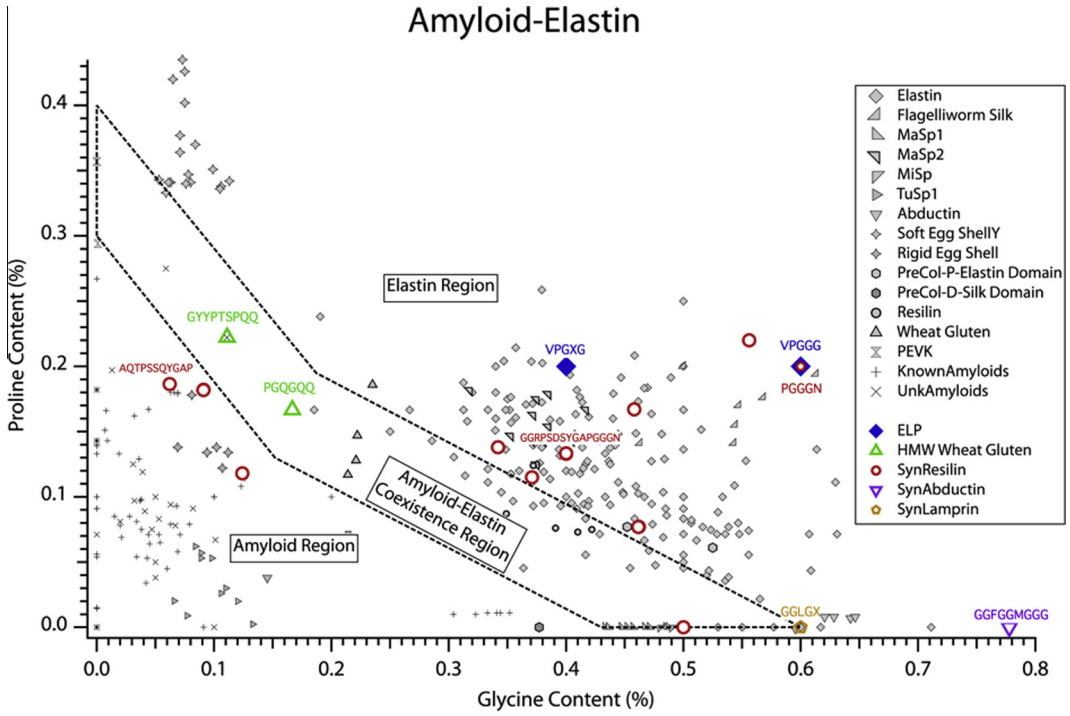

Fig. 3. Tandem repeat proteins plotted as a function of % proline & % glycine. This figure, adopted from [61], surveys a large number of elastin and amyloid forming proteins to help predict the propensity of a protein to form amyloid fibrils based solely on the proline and glycine content of the peptide chain. The dotted line region represents a transitional composition between elastic and amyloid regions. Their model supports a unified model of protein aggregation where conformation disorder and chain hydration are paramount and shows clear evidence supporting proline and glycine roles in promoting disorder in ELPs. Plotted here are several bioinspired repeat proteins that have some degree of elasticity. An interesting observation is the structural and compositional similarity of ELP and lamprin, both predicted to be elastomeric, yet sampling dramatically different conformation space (lamprin adopts beta sheet under physiologic conditions). Also remarkable is the vast sequence space of resilin, the only protein that spans the amyloid–elastin divide. Clearly, proline/glycine content is not the only factor in determining disorder and this graph further provides evidence that zwitterionic charge and perfect tandem repeat protein chains may also be contributing variables.

separation [61]. With 20% proline and 60% glycine, the pentapeptide building block of ELPs represents a near perfect elastomeric protein; however, this feature does not fully exclude ELPs from the propensity to form amyloids. Study of several of the isolated exons from tropoelastin by Keeley has shown that even elements of tropoelastin, a protein that depends so readily on maintaining elastic characteristics, will assemble into amyloid fibers upon aggregation [65,66].

The study of amyloid fiber formation is a critical aspect of the IDP field, as predominantly disordered sequences are implicated in amyloid/prion formation leading to neurodegenerative disorders such as Alzheimer's and Huntington's disease [67]. It has been shown that for some prion-forming proteins, randomization of the amino acid sequence does not inhibit amyloid formation, that sequence alone is not strongly predictive of the propensity to amyloid formation, but that features other than sequence likely control aggregation. As elastomeric proteins exist on a spectrum from amyloids to fuzzy aggregates they offer an interesting perspective on the propensity of other amino acid sequences to contribute to the formation of amyloids. Simple sequence modifications to ELPs and the inclusion of guest residues such as glutamine and asparagine, often present in prions, may offer a useful model system to better study the impact of sequence constraints on amyloid formation in an artificial protein polymer.

3.3. Phase behavior in biological systems

There is growing evidence that suggests that phase separation of multivalent, low-complexity proteins is essential for the regulation of intracellular protein–protein and protein–RNA assemblies [28,68,69]. Brangwynne et al. found that germline processing (P) granules in *Caenorhabditis elegans* undergo a transition between a soluble and a condensed globule phase and suggested that phase separation and localization of these granules within the cytoplasm represents a mechanism for cytoplasmic organization in the developing embryo [70]. The use of recombinant proteins to study phase transitions in cells has also been reported. Kato et al. observed that low-complexity sequences are both necessary and sufficient for eukaryotic RNA granule formation [71]. One LC domain in particular, the fused in sarcoma (FUS) RNA binding protein, is capable of concentration dependent formation of hydrogels that retain the LC domains of other RNA binding proteins [71], hinting at the importance of phase behavior and IDPs in controlling protein translation.

Li et al. produced recombinant proteins containing SH3 domains or their proline-rich interaction partner (PRM) and demonstrated a liquid–liquid phase separation of these polymers at sufficiently high concentration and ligand valency [72]. They also showed that this type of multivalency triggered aggregation could be used to control actin assembly using the naturally occurring NCK-nephrin-N-WASP protein system [72]. There are many other examples of IDPs involved in intracellular phase transitions, and we point readers to a recent review by Toretsky and Wright for a more detailed examination of this fascinating phenomenon [68].

A critical distinction between this type of regulatory phase behavior and amyloid fiber formation is concentration dependent reversibility. Although several of these IDPs appear to form amyloid type structures [71], none of them adopt permanent misfolded structures such as those found in prions. The connection between the phase behavior in an elastin-based system to other biological systems may not immediately seem relevant, as the phase behavior of ELPs is temperature dependent, while the temperature in live

organisms is constant. However, consistent with polymer physics theory for cloud point transitions [73], an ELP (of defined length and composition) can be thought of as existing on a two-axis phase diagram where the aggregation, or spinodal decomposition, is dependent on both temperature and concentration. Experimental evidence has shown that an ELP's aggregation temperature scales with the logarithm of concentration [9,41], so that changes in concentration can in fact be a trigger to isothermally drive the phase transition of an ELP. ELPs can also be designed to isothermally phase separate in response to changes in pH [13,14]. This parameter offers an additional level of control that could be important given the pH variability in the subcellular microenvironment. As phase separation of ELPs and these cellular systems are both based on the prevalence of low sequence complexity disorder and reversible aggregation, understanding the sequence level determinants that drive phase behavior in ELPs can, we believe, be helpful in understanding the rules for the design of artificial systems are capable of subcellular compartmentalization. In fact, the concept of subcellular localization was recently applied to an ELP-based system by both Huber et al., who used elastin domains as the building blocks for intracellular "organelle-like" compartments in *Escherichia coli* [74], and by Pastuszka et al., who used the phase separation of ELP-clathrin light chain fusions to thermally control clathrin-mediated endocytosis [75].

4. Biomedical utility of ELPs

The intrinsic disordered properties that make ELPs an attractive model for IDPs have also been an essential component of their widespread use. In particular, the mechanical elastic recoil and tunable phase behavior have lent themselves remarkable well to functionality in biomedical and materials engineering (Fig. 4). Given the similarities between ELPs and other IDPs, we propose that derivatives of other IDPs may find similar biomedical applications as those discussed for ELPs. A complete consideration of the biomaterial engineering potential of ELPs is outside of the scope of this review, and for a more comprehensive analysis, we direct the readers to a review of the applications of ELPs [76], and more specific reviews on the use of ELPs for drug delivery [77], and tissue engineering [25].

ELPs have been extensively developed as carriers for drug delivery, as their stimuli-responsive behavior, lack of toxicity, and tunable half-life in systemic circulation provide useful attributes for the delivery of drugs. With these attributes in mind, we have developed several highly potent chemotherapeutic-loaded ELP nanoparticles for cancer therapy. In one approach, an ELP is designed with a short peptide tag at one of its termini that contains multiple copies of Cys residues separated by a diglycine spacer. Attachment of multiple copies of a hydrophobic cancer drug to the Cys residues then spontaneously triggers the self-assembly of the ELP into near-monodisperse micelles with size ranging between 40 and 60 nm. ELP-based micelles containing multiple copies of doxorubicin (Dox) was shown to be highly potent in eradicating colon carcinoma in a murine model even as a single injection [22]. More recently, this approach has been extended to the delivery of paclitaxel, another widely used chemotherapeutic in the clinic [78].

These designs of drug-loaded ELP nanoparticles did not exploit the thermal responsiveness of ELPs. In a subsequent study, McDaniel et al. showed that the ELP micelle's thermo-responsive properties can be predicted de novo (by choice of the guest residue X in the VPGXG repeat unit and the molecular weight) allowing for

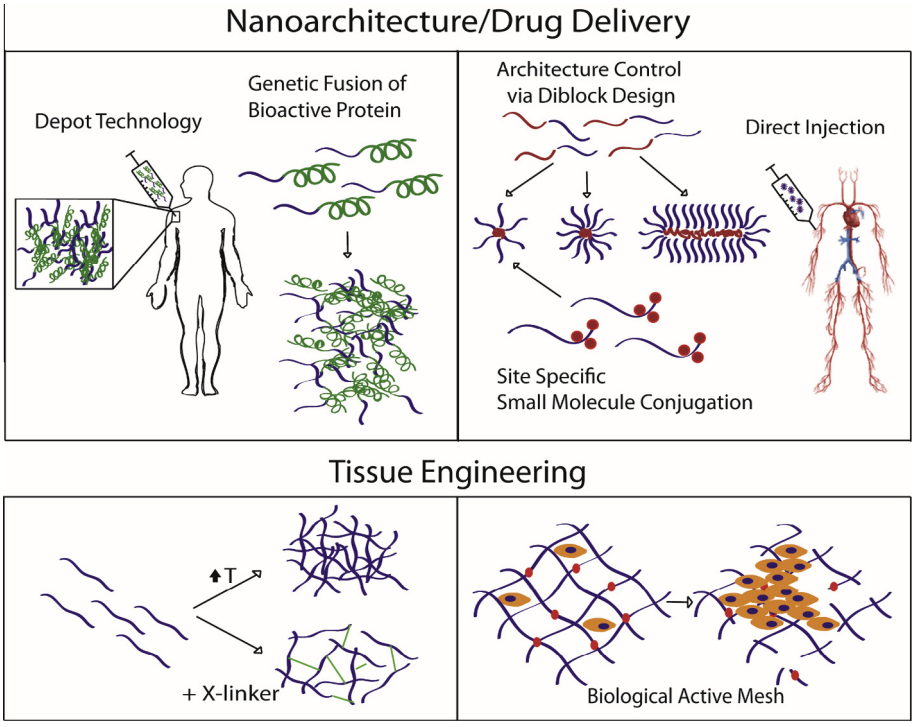

Fig. 4. Biomedical applications of elastin-like-polypeptides (ELPs). For the past decade, ELPs have been utilized for a variety of biomedical applications. These applications can broadly be broken down into two categories: exploiting the biophysical properties of ELPs for drug delivery and for tissue engineering. Researchers have utilized the phase behavior of ELPs to make injectable depots for sustained glucose control [24] and have utilized the unique self-assembly properties of protein amphiphiles for delivery of cancer therapeutics [22,77]. In tissue engineering, researchers have cleverly designed physically and chemically cross-linked hydrogels to support tissue growth and have designed next generation scaffolds that are precisely engineered for cell attachment and proteolytic degradation [25,73,80]. All of these applications require a fundamental understanding of an ELP's sequence effect on the transition temperature, which was elucidated through systematic study [8,9]. Other repeat proteins could provide a new set of materials for biomedical application if their biophysical properties were equally understood.

simple design of a nanoparticle that possesses a physiologically relevant nanoparticle-to-aggregate transition temperature. These nanoparticles were shown to aggregate in the vasculature of tumors that were externally heated to 42 °C, a temperature that is clinically compatible, typically in combination with external beam radiation as a treatment modality. In a different design of ELPs, diblock ELPs have been designed with two distinct segments that are covalently linked, where one block has greater hydrophobicity than the other block. Below a critical micellization temperature (CMT), these diblock ELPs are soluble, but as the temperature is raised above the CMT, the more hydrophobic block selectively desolvates, thereby making the diblock amphiphilic enough to self-assemble into spherical micelles. These nanoparticles have been used in vitro to trigger the formation of nanoparticles for the multivalent display of cell penetrating peptide motifs for controlled cellular uptake and in vivo for the thermal targeting of drug to tumors heated to 42 °C [21,79]. In the latter case, subsequent heating and cooling of the in vivo area of interest created a "thermal pump" capable of continually creating a diffusion gradient of the drug across the tumor vasculature.

Sustained release delivery systems have also been developed that consist of the fusion of peptide and protein drugs to soluble ELPs that undergo phase separation in vivo. This approach has been used for the controlled release of peptide drugs from subcutaneous depots for the treatment of type 2 diabetes [24]. Amiram et al. genetically fused glucagon-like peptide-1 to an ELP with a phase transition below physiological temperature. When injected under the skin, the polymer formed an insoluble coacervate, which slowly released the GLP1-ELP fusion over time, controlling glucose levels in mice for up to 5 days with a single injection.

Given the importance of tropoelastin in the extracellular matrix of many tissues, it is no surprise that ELPs have been used as cell scaffolds for tissue engineering. The properties of stimuli-responsiveness, biocompatibility, and biodegradation of ELPs are attractive as tissue scaffolds particularly because these parameters are difficult to control and combine into a single system. Different applications of tissue engineering require materials with different levels of crosslinking density, molecular weight and concentration to provide combination of microstructure and mechanical properties. Trabbic-Carlson et al. showed that the stiffness of cross-linked ELP hydrogels could be tuned from 1.6 to 15 kPa at 37 °C by controlling the MW and crosslink density of the ELPs. Taking advantage of the genetic encoding capability of ELPs to regularly introduce crosslinking junctions and specifically determine the peptide MW has allowed ELPs to distinguish themselves as ideal scaffolds for a variety of applications including cartilage, intervertebral disc, vascular graft, liver, ocular and cell sheet engineering [25]. However, the genetic encoding of ELPs also confers the ability to incorporate bioactive residues that can both interact with different cellular types and also control degradation of the scaffold [73]. In both cases, these provide better biomimicry of the natural ECM. One of the simplest examples is the incorporation of RGD binding domains into ELP matrices for cell adhesion [80].

RGD motifs have become the gold standard of cellular attachment in the tissue engineering literature due to their small size, yet significant biological activity. As the field of IDPs has expanded, it has become clear that many other short, specific sequences exist that play important biological roles. These short linear motifs (SLiMs) are implicated in a diverse array of protein interactions including enzyme recruitment, protein trafficking, and protein complex assembly [29]. With low affinity but high specificity, disordered SLiMs are a very attractive for inclusion in elastomeric polymer materials. As they can be encoded at the genetic level without total disruption of ELP material properties, their controlled inclusion in ELP based systems is a clear new direction.

5. Other elastomeric polymers with IDP characteristics

The backbone sequence and repetitive nature of ELPs plays a critical role in the chain conformations they sample in solution. However, elastin-derived repeat proteins are just one example of an elastomeric tandem repeat protein. Searching the literature for proteins with similar features – largely unstructured proteins that are rich in proline and glycine and with a highly conserved sequence across species – offers a multitude of other IDPs with a vast array of natural functions. To date, they have been differentiated based on their origin and their macroscopic material properties (i.e. elastin, resilin, collagen, byssus threads et cetera). Herein, we argue that these diverse materials should instead be thought of as members of a larger family of intrinsically disordered tandem repeat proteins, where the organization and content of amino acids leads to their biological function and hierarchical material properties. An interdisciplinary research approach that spans materials science and the biophysics and structural biology community that focuses on specific questions relevant to IDPs—such as the effect of proline and glycine content on elastomeric properties presented in Fig. 3—would provide reciprocal insights into the biological functions of IDPs and the design of novel biomaterials.

5.1. A comparison of abductin, resilin, lamprin and HMW wheat gluten

Abductin, resilin and high molecular weight (HMW) wheat gluten are three proteins that most resemble tropoelastins (and ELPs) based on their sequence [81]. Each protein contains long domains of elastomeric repeat units, interspersed with non-repetitive units. Abductin is a naturally cross-linked elastomer that serves as the primary structural component for bivalves in mollusks, and is characterized by high resilience to compression ratio and a consensus repeat sequence of GGFGGMGGGX--where X can be any amino acid [82]. Resilin is an elastomeric protein predominantly found in Drosophila melanogaster (fruit fly), Anopheles gambiae (mosquito), and arthropod cuticles, where fast, repetitive motion and efficient energy storage is required [83]. Depending on its origin, resilin has a consensus repeat sequences of GGRPSDSYGAPGGGN or AQTPSSQYGAP [36,84]. Lamprin (GGLGX) [85], the most important protein in lamprey cartilage, has been shown to exhibit elastomeric properties [86]. The high molecular weight (HMW) subunits of wheat gluten are seed storage proteins that store essential nutrients such as carbon, nitrogen and sulfur for growth of seedlings. Their consensus sequences are the hexapeptide repeat PGQGQQ and the non-apeptide repeat GYYPTSPQQ. Elastin, abductin and resilin utilize crosslinking domains (disulfides in abductin, dityrosines in resilin) to form mechanically elastic gels [87].

Evaluation of their consensus repeats by their charge/hydropathy balance suggests that abductin and lamprin fall in the molten globule region with ELPs, HMW gluten and resilins are predicted to be disordered. This result is expected as abductin and lamprin have repeat sequences most homologous to ELPs. However, NMR, CD, and Fourier Transform Infrared Spectroscopy (FTIR) analysis of short, repeat units of the connecting elastic domains of HMW gluten, resilin and abductin indicate that they are all largely disordered with each displaying varying levels of poly-proline helix (PPII) and β-turn structures, similar to ELPs. Fig. 3 shows these and other artificial repeat proteins on the traditional amyloid–elastin graph, where the properties of these proteins can predicted—albeit to a limited extent—by their proline and glycine content. An interesting observation requiring further investigation is the close sequence relationship between lamprin and ELP, though lamprin, unlike ELPs, can adopt a β-sheet conformation in aqueous solution. Their biophysical similarity is noteworthy given the disparity in their primary sequence and composition, especially between elas-

tin and resilin/HMW gluten [88]. This leads us to the questions, "What is the cause for this similarity?" and "How we may better attribute their differences in sequence to differences in solution properties?" Although each unit (besides lamprin) is disordered, they seem to have used different combinations of amino acids to achieve disorder. For resilin and HMW wheat gluten, the chains utilize zwitterionic charge and hydrogen bonding to water to prevent aggregation. On the other hand, abductin and ELP do not utilize these principles. ELPs in particular, achieve disorder by structure breaking proline and glycine residues that enable sampling of many possible conformation conformations. Abductin, interestingly exhibits chain disorder without regularly spaced structure breaking proline residues. These examples, when assessed as members within the larger family of IDPs, highlight the challenges that exist when it comes to predicting disorder of protein sequences containing repeated motifs.

5.2. Aggregation and phase behavior

In the IDP community, there is great interest in phase transitions of IDP and IPDRs in aqueous solvent. ELPs are the canonical artificial protein polymers that exhibit LCST phase behavior but such aqueous phase behavior is not restricted to ELPs. Polymers derived from other peptide motifs in elastin, resilin, and abductin all display upper critical solution temperature (UCST) or LCST phase behavior, despite their highly divergent sequences. Interestingly, abductin and resilin-like polypeptides exhibit both an UCST and a LCST [89,90]. Additionally, modest changes in sequence and composition can affect the phase behavior. This effect has been well characterized in ELPs [8,9] while similar effects have been seen on the LCST of abductin and the LCST of resilin [91,92]. Li et al. have explored the LCST behavior of resilin-like repeat proteins and shown that their aggregation is a function of concentration and also displays interesting hysteretic behavior upon subsequent cooling [92]. However, without the precise control of length, sequence and composition of resilin and abductin-based proteins, it is difficult to predict how exactly one could tailor this behavior to a specific application. The implication that the protein sequence determines the presence and temperature at which UCST or LCST behavior presents unique opportunities for engineering for biomedical engineering or materials to create next generation stimuli responsive materials. Further investigation of longer repeat units of HMW wheat gluten and lamprin will likely yield polymers that exhibit phase behavior in the experimentally observable 0–100 °C range based on their sequence homology to resilin and abductin respectively.

The importance of sequence does not end with the solution properties of these repetitive proteins. The most extensively studied systems are composed of elastin, silk/collagen or resilin but a striking example by Bochicchio et al. demonstrates the importance of molecular weight and specific amino acids (phenylalanine) on the formation of honeycomb gels and the importance of the peptide sequence on the morphology of physically cross-linked abductin structures [91]. ELP based hydrogels also display these types of sequence dependent material properties, as several studies have shown that the amino acid sequence can predict the reversible swelling and collapsing temperature of the resulting chemically cross-linked hydrogel [19,93].

ELPs also retain their phase behavior upon crosslinking, exhibited as a stiffening of the cross-linked ELP network with an increase in solution temperature [19]. Similar to ELP networks, resilin-like polypeptides (RLPs) also have decreased swelling properties with increasing crosslinking density and similar dynamic moduli [94]. However, RLP hydrogels have diffusion constants (D) that are one order of magnitude higher than ELPs hydrogels (D in the range 10^{-8} cm^2 s^{-1} with hydrogel crosslink density of 14.4 mol cm^{-3}) reported by Truong et al. and Lee et al. [93,94], indicating a difference in pore size for these structures. These differences must be treated with caution as the materials processing parameters used to obtain the ELP and RLP gels could have a significant impact on the properties of the cross-linked materials, independent of their composition and sequence. Additionally, the dynamic modulus of RLP hydrogels is oddly not a function of temperature [94]. The sequence of RLPs appears to have an impact on the properties of the cross-linked materials but it is challenging to tease out specific information for each residue without a systematic approach to controlling monomer molecular weight, sequence and crosslink density. For more details on the effect of sequence on bulk mechanical properties of cross-linked hydrogels of this class of materials, readers are directed to references [25,90,95–97] for more details.

5.3. Combinatorial protein materials in nature

Mussel byssus threads and silks have all been classified as elastomeric proteins because of their J shaped stress strain curves, indicative of a molecular level chain organization that endows them with the stretch and relaxation behavior of elastic polymers [6]. Despite this macroscale characterization, each of these natural materials is more complicated at the sequence level than ELPs and tropoelastin despite containing multiple regions of tandem repeat units and glycine and proline rich regions. In both materials, elastin-like sequences are interspersed between other unique repeat motifs. Therefore, assigning a role for the unique mechanical behavior of silk and byssus threads at the sequence level is very difficult but analyzing the protein backbone for patterns yields some useful insights:

1. Multiple, tandem repeat regions contribute to the material and biophysical properties of IDPs. However, close analysis of these regions shows that these regions are not completely conserved in each IDP. In fact, the same repeat regions (i.e. proline–glycine rich regions in elastin) are utilized in different IDPs. However, the density (# repeat instances/chain length), compositional percentage (length of the sum of repeat motifs/length protein) and organization (surrounding motifs, N or C termini clustering et cetera) changes drastically, giving rise to unique functions to different IDPs.
2. Recombinant synthesis and characterization of different domains can provide insights into how the full length protein generates their unique mechanical behaviors.

Despite the interest in water-proof adhesive materials and extensive cDNA information of various mussel byssus threads, recombinant reconstruction of these materials remains a challenge [98]. In byssus threads, over a dozen proteins come together to make the byssus structure but the most interesting pair are the PreCols that extend along the majority of the fibril. PreCol D, which is dominant at the distal end of the thread and decreases in abundance toward the proximal end, has flanking domains that resemble a motif sequence of spider dragline silk. PreCol P, which exists in a gradient complementary to PreCol D, has flanking regions that closely resemble elastin, and PreCol NG, which is uniformly present throughout the thread, has Gly-rich flanking domains that resemble plant cell wall proteins [99–101]. The collagen domains along this backbone are highly post-translationally modified from proline to Hydroxyproline and Tyr 3,4-dihydoxyphenylalanine (DOPA) and have drastically different mechanical properties that evolution optimized to protect the mussel from catastrophic thread failure [102]. At the end of these fibers, small repeats of the complex domain thought to be responsible for binding to the substrate (AKPSTPPTYK) have random coil conformations that spontaneously

form fibril aggregates upon post-translational modification of tyrosine to DOPA, which highlights the importance of these residue modifications to the structure of this protein [103]. Even though extensive analysis of three whole mussel foot protein regions was completed in 2012, each combining unique repeat sequences with varying degrees of intrinsic disorder present in the parent protein [104], researchers have still failed to reproduce this tough biomaterial's properties of robust, wetted adhesion [100].

Silk proteins display the incredible ability of combining the same repeat domains in different ways to produce vastly different materials with different properties: GPGGX/GPGQQ, GGX, Poly-A or Poly-GA and a spacer sequence [105]. Hayashi & Lewis hypothesized a different function for these repeat units [105]. He suggested that the GPGGX/GPGQQ repeat units resemble unstructured elastin domains, Poly-A/GA repeats form beta sheets to form crystalline regions with the alpha helical Poly-GGA domains, and random regions that he hypothesized help impart chain flexibility and solubility. In each case, the unique repeat regions were analyzed independently for secondary structure, which suggested their function in the parent material. These insights stimulated the design of a range of recombinant biomaterials [106]. Recently, recombinant expression of polymers consisting of short sequences inspired by garden spider spidroins that were processed by a biomimetic methodology resulted in materials with the same fiber toughness as natural silks [107]. This example of reconstruction of silk by first understanding its individual components is reminiscent of ELP/tropoelastin. Recapitulation of the properties of tropoelastin from its domains elucidated the role different molecular motifs that endow tropoelastin with its interesting mechanical behavior [108]. In each instance, a sequence-centric approach to studying the structure characteristics of each domain was critical to reproducing the mechanical behavior of elastin. Therefore we advocate that a similar approach should be employed to study other IDP or IDPRs, especially multidomain IDPs that form prions or amyloid fibers.

6. Discussion

6.1. Understanding IDPs by analysis of ELPs

Given the rapid expansion of research on IDPs, it is important to establish a model protein to which other sequences can be compared. We suggest that ELPs, protein polymers that are based on perfect tandem repeats of the consensus hydrophobic domain of elastin, are one such class of proteins. Fundamental to the disordered properties of ELPs are the perfect dispersion of proline and glycine motifs, precluding the development of secondary structure but allowing transient structural sampling, and minimal hydrophobicity [61]. These properties not only keep ELP disordered in a solvated state, but also allow retention of disorder upon aggregation. This "fuzzy" aggregation has been observed to varying degrees with a number of IDPs and has also been likened to the highly reversible binding properties of other IDPs [62].

We have systematically shown that small changes in sequence lead to exquisite control of the aggregation tendencies of ELPs, and that larger disruptions to the sequence, as seen in other elastomeric IDPs, can lead to new polymer behavior such as UCST phase behavior or fiber formation. In the case of ELPs, control over phase behavior has been critical to their widespread use in tissue engineering and drug delivery applications. However, a serendipitous outcome of this drive is a set of sequence rules that explains how amino acids control the propensity of a disordered chain to self-aggregate. This phenomenon is a huge area of investigation within the world of IDPs and IDPRs given the emerging knowledge on subcellular regulation controlled by phase separation and the long-standing problem of amyloid formation in neurodegenerative diseases [67]. Given the extensive understanding of ELPs, the careful study of larger and more complex IDPs/IDPRs using some of the same methodologies employed for ELPs will yield new insights into these systems.

6.2. Protein material design

Based on our knowledge of the relationship of phase behavior and disorder in ELPs, it is a reasonable hypothesis that other intrinsically disordered proteins may also be capable of similar phase behavior. In fact, this property has already been noted for several mechanically active, elastomeric domains, including resilin and abductin, which are capable of UCST, LCST, or a combination of the two behaviors [36,88,92]. When considering the design of combinatorial materials with ELPs, an obvious first step is the inclusion of similar elastomeric domains in either mixtures or encoded together at the sequence level. Nature has already provided several examples of this type of material in silk and byssus threads [6], but our analysis suggests that precise sequential control over these elastomeric domains would lead to the development of protein materials with precisely engineered mechanical properties.

Although combinations of elastomeric domains is a first logical step, the prevalence of disorder in a host of biologically active protein–protein and protein–RNA interactions implies that other systems could be created that take advantage of the combination of biological and material properties. There has been a significant body of work describing the biological utility of disordered SLiMs [29]. Their function, particularly in multivalent cellular systems, suggest that a variety of protein based materials would benefit from incorporation of SLIMs into these materials, leading to the design of new bioactive materials.

7. Conclusions

The field of intrinsically disordered proteins has rapidly expanded in the last few decades with the discovery of numerous proteins with biological function that are largely structurally disordered. While the precise amino acid sequences of these disordered proteins varies based on their biological functionality, they all share common sequence traits that encode disorder. ELPs are an idealized and minimal family of IDPs whose disorder in both their solvated and aggregated states is driven by low-complexity, tandem repeats that create disordered structures whose solvation is temperature dependent and which imparts a soluble–insoluble LCST phase behavior to these protein polymers. The origins of the extensive biophysical study of ELPs stems from their ability to recapitulate the LCST phase behavior of tropoelastin and by the elasticity exhibited by cross-linked ELPs. More recently, the stimulus for the exploitation of their phase behavior has been driven by many molecular applications of this class of polymers.

This review seeks to bridge the cultural and scientific gap between researchers working on artificial protein polymers and those studying native IDPs. The analysis of IDPs through the lens of ELPs and other protein polymers, and conversely the design of low sequence complexity protein polymers, would yield a significantly greater physical understanding of their biophysical properties and how modification of those properties would affect function. Additionally, the recognition that many IDPs share structural features with protein polymers suggests a potentially rich line of investigation of the design of new macromolecules that combine features of artificial protein polymers with IDPs for specific biological or material functions. We hope that the relationship of ELPs to IDPs elucidated here will lead to future exploration of how cross-talk between the fields of recombinant peptide polymers and IDPs can help push both fields forward.

References

[1] Neuman, R. and Logan, M. (1973) The determination of collagen and elastin in tissues. J. Biol. Chem. 186, 549–556.
[2] Bergel, D.H. (1961) The static elastic properties of the arterial wall. J. Physiol. 156, 445–457.
[3] Vrhovski, B. and Weiss, A.S. (1998) Biochemistry of tropoelastin. Eur. J. Biochem. 258, 1–18.
[4] Gray, W.R., Sandberg, L.B. and Foster, J.A. (1973) Molecular model for elastin structure and function. Nature 246, 461–466.
[5] Meyers, M.A., Chen, P.Y., Lin, A.Y.M. and Seki, Y. (2008) Biological materials: structure and mechanical properties. Prog. Mater Sci. 53, 1–206.
[6] Gosline, J., Lillie, M., Carrington, E., Guerette, P., Ortlepp, C. and Savage, K. (2002) Elastic proteins: biological roles and mechanical properties. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357, 121–132.
[7] Urry, D.W., Trapane, T.L. and Prasad, K.U. (1985) Phase-structure transitions of the elastin polypentapeptide-water system within the framework of composition-temperature studies. Biopolymers 24, 2345–2356.
[8] Meyer, D.E. and Chilkoti, A. (2002) Genetically encoded synthesis of protein-based polymers with precisely specified molecular weight and sequence by recursive directional ligation: examples from the elastin-like polypeptide system. Biomacromolecules 3, 357–367.
[9] McDaniel, J.R., Radford, D.C. and Chilkoti, A. (2013) A unified model for de novo design of elastin-like polypeptides with tunable inverse transition temperatures. Biomacromolecules 14, 2866–2872.
[10] Cho, Y., Zhang, Y., Christensen, T., Sagle, L.B., Chilkoti, A. and Cremer, P.S. (2008) Effects of hofmeister anions on the phase transition temperature of elastin-like polypeptides. J. Phys. Chem. B. 112, 13765–13771.
[11] Christensen, T., Hassouneh, W., Trabbic-Carlson, K. and Chilkoti, A. (2013) Predicting transition temperatures of elastin-like polypeptide fusion proteins. Biomacromolecules 14, 1514–1519.
[12] Trabbic-Carlson, K., Meyer, D.E., Liu, L., Piervincenzi, R., Nath, N., LaBean, T. and Chilkoti, A. (2004) Effect of protein fusion on the transition temperature of an environmentally responsive elastin-like polypeptide: a role for surface hydrophobicity? Protein Eng. Des. Sel. 17, 57–66.
[13] Girotti, A., Reguera, J., Arias, F.J., Alonso, M., Testera, A.M. and Rodríguez-Cabello, J.C. (2004) Influence of the molecular weight on the inverse temperature transition of a model genetically engineered elastin-like pH-responsive polymer. Macromolecules 37, 3396–3400.
[14] MacKay, J.A., Callahan, D.J., FitzGerald, K.N. and Chilkoti, A. (2010) Quantitative model of the phase behavior of recombinant pH-responsive elastin-like polypeptides. Biomacromolecules 11, 2873–2879.
[15] Strzegowski, L.A., Martinez, M.B., Gowda, D.C., Urry, D.W. and Tirrell, D.A. (1994) Photomodulation of the inverse temperature transition of a modified elastin poly(pentapeptide). J. Am. Chem. Soc. 116, 813–814.
[16] McDaniel, J.R., Weitzhandler, I., Prevost, S., Vargo, K.B., Appavou, M.-S., Hammer, D.A., Gradzielski, M. and Chilkoti, A. (2014) Noncanonical self-assembly of highly asymmetric genetically encoded polypeptide amphiphiles into cylindrical micelles. Nano Lett. 14, 6590–6598.
[17] Wright, E.R. and Conticello, V.P. (2002) Self-assembly of block copolymers derived from elastin–mimetic polypeptide sequences. Adv. Drug Deliv. Rev. 54, 1057–1073.
[18] Dreher, M.R., Simnick, A.J., Fischer, K., Smith, R.J., Patel, A., Schmidt, M. and Chilkoti, A. (2008) Temperature triggered self-assembly of polypeptides into multivalent spherical micelles. J. Am. Chem. Soc. 130, 687–694.
[19] Trabbic-Carlson, K., Setton, L.A. and Chilkoti, A. (2003) Swelling and mechanical behaviors of chemically cross-linked hydrogels of elastin-like polypeptides. Biomacromolecules 4, 572–580.
[20] Martín, L., Alonso, M., Girotti, A., Arias, F.J. and Rodríguez-Cabello, J.C. (2009) Synthesis and characterization of macroporous thermosensitive hydrogels from recombinant elastin-like polymers. Biomacromolecules 10, 3015–3022.
[21] MacEwan, S.R. and Chilkoti, A. (2012) Digital switching of local arginine density in a genetically encoded self-assembled polypeptide nanoparticle controls cellular uptake. Nano Lett. 12, 3322–3328.
[22] MacKay, J.A., Chen, M., McDaniel, J.R., Liu, W., Simnick, A.J. and Chilkoti, A. (2009) Self-assembling chimeric polypeptide–doxorubicin conjugate nanoparticles that abolish tumours after a single injection. Nat. Mater. 8, 993–999.
[23] Bidwell, G.L. and Raucher, D. (2005) Application of thermally responsive polypeptides directed against c-Myc transcriptional function for cancer therapy. Mol. Cancer Ther. 4, 1076–1085.
[24] Amiram, M., Luginbuhl, K.M., Li, X., Feinglos, M.N. and Chilkoti, A. (2013) Injectable protease-operated depots of glucagon-like peptide-1 provide extended and tunable glucose control. Proc. Natl. Acad. Sci. 110, 2792–2797.
[25] Nettles, D.L., Chilkoti, A. and Setton, L.A. (2010) Applications of elastin-like polypeptides in tissue engineering. Adv. Drug Deliv. Rev. 62, 1479–1485.
[26] Betre, H., Setton, L.A., Meyer, D.E. and Chilkoti, A. (2002) Characterization of a genetically engineered elastin-like polypeptide for cartilaginous tissue repair. Biomacromolecules 3, 910–916.
[27] Tompa, P. and Ferst, A. (2009) Structure and Function of Intrinsically Disordered Proteins, Chapman & Hall/CRC Press, Boca Raton, FL.
[28] van der Lee, R., Buljan, M., Lang, B., Weatheritt, R.J., Daughdrill, G.W., Dunker, A.K., Fuxreiter, M. and Babu, M.M. (2014) Classification of intrinsically disordered regions and proteins. Chem. Rev. 114, 6589–6631.
[29] Van Roey, K., Uyar, B., Weatheritt, R.J., Dinkel, H., Seiler, M., Budd, A., Gibson, T.J. and Davey, N.E. (2014) Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. Chem. Rev. 114, 6733–6778.
[30] Dunker, A.K., Cortese, M.S., Romero, P., Iakoucheva, L.M. and Uversky, V.N. (2005) Flexible nets: the roles of intrinsic disorder in protein interaction networks. FEBS J. 272, 5129–5148.
[31] Pappu, R.V., Wang, X., Vitalis, A. and Crick, S.L. (2008) A polymer physics perspective on driving forces and mechanisms for protein aggregation. Arch. Biochem. Biophys. 469, 132–141.
[32] Uversky, V.N. (2013) A decade and a half of protein intrinsic disorder: Biology still waits for physics. Protein Sci. 22, 693–724.
[33] Sickmeier, M., Hamilton, J.A., LeGall, T., Vacic, V., Cortese, M.S., Tantos, A., Szabo, B., Tompa, P., Chen, J., Uversky, V.N., Obradovic, Z. and Dunker, A.K. (2007) DisProt: the database of disordered proteins. Nucleic Acids Res. 35, D786–D793.
[34] Uversky, V.N., Gillespie, J.R. and Fink, A.L. (2000) Why are "natively unfolded" proteins unstructured under physiologic conditions? Proteins Struct. Funct. Bioinform. 41, 415–427.
[35] Li, B., Alonso, D.O. and Daggett, V. (2001) The molecular basis for the inverse temperature transition of elastin. J. Mol. Biol. 305, 581–592.
[36] Balu, R., Dutta, N.K., Choudhury, N.R., Elvin, C.M., Lyons, R.E., Knott, R. and Hill, A.J. (2014) An16-resilin: an advanced multi-stimuli-responsive resilin–mimetic protein polymer. Acta Biomater. 10, 4768–4777.
[37] Fuxreiter, M. and Tompa, P. (2012) Fuzziness: Structural Disorder in Protein Complexes, Springer Science & Business Media, New York, NY.
[38] Elvin, C.M., Carr, A.G., Huson, M.G., Maxwell, J.M., Pearson, R.D., Vuocolo, T., Liyou, N.E., Wong, D.C.C., Merritt, D.J. and Dixon, N.E. (2005) Synthesis and properties of crosslinked recombinant pro-resilin. Nature 437, 999–1002.
[39] Jorda, J., Xue, B., Uversky, V.N. and Kajava, A.V. (2010) Protein tandem repeats – the more perfect, the less structured. FEBS J. 277, 2673–2682.
[40] Das, R.K. and Pappu, R.V. (2013) Conformations of intrinsically disordered proteins are influenced by linear sequence distributions of oppositely charged residues. Proc. Natl. Acad. Sci. 110, 13392–13397.
[41] Meyer, D.E. and Chilkoti, A. (2004) Quantification of the effects of chain length and concentration on the thermal behavior of elastin-like polypeptides. Biomacromolecules 5, 846–851.
[42] Hoeve, C.A.J. and Flory, P.J. (1974) The elastic properties of elastin. Biopolymers 13, 677–686.
[43] Aaron, B.B. and Gosline, J.M. (1981) Elastin as a random-network elastomer: a mechanical and optical analysis of single elastin fibers. Biopolymers 20, 1247–1260.
[44] Gosline, J.M. (1978) Hydrophobic interaction and a model for the elasticity of elastin. Biopolymers 17, 677–695.
[45] Urry, D.W., Hugel, T., Seitz, M., Gaub, H.E., Sheiba, L., Dea, J., Xu, J. and Parker, T. (2002) Elastin: a representative ideal protein elastomer. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357, 169–184.
[46] Debelle, L. and Tamburro, A.M. (1999) Elastin: molecular description and function. Int. J. Biochem. Cell Biol. 31, 261–272.
[47] Lelj, F., Tamburro, A.M., Villani, V., Grimaldi, P. and Guantieri, V. (1992) Molecular dynamics study of the conformational behavior of a representative elastin building block: Boc-Gly-Val-Gly-Gly-Leu-OMe. Biopolymers 32, 161–172.
[48] Kurková, D., Kríz, J., Schmidt, P., Dybal, J., Rodríguez-Cabello, J.C. and Alonso, M. (2003) Structure and dynamics of two elastin-like polypentapeptides studied by NMR spectroscopy. Biomacromolecules 4, 589–601.
[49] Isailovic, D., McMillan, R.A. and Conticello, V.P. (2003) Structure of an elastin–mimetic polypeptide by solid-state NMR. Biopolymers 70, 158–168.
[50] Pometun, M.S., Chekmenev, E.Y. and Wittebort, R.J. (2004) Quantitative observation of backbone disorder in native elastin. J. Biol. Chem. 279, 7982–7987.
[51] Perry, A., Stypa, M.P., Tenn, B.K. and Kumashiro, K.K. (2002) Solid-state ^{13}C NMR reveals effects of temperature and hydration on elastin. Biophys. J. 82, 1086–1095.
[52] Wright, E.R., McMillan, R.A., Cooper, A., Apkarian, R.P. and Conticello, V.P. (2002) Thermoplastic elastomer hydrogels via self-assembly of an elastin–mimetic triblock polypeptide. Adv. Funct. Mater. 12, 149–154.
[53] Reiersen, H., Clarke, A.R. and Rees, A.R. (1998) Short elastin-like peptides exhibit the same temperature-induced structural transitions as elastin polymers: implications for protein engineering. J. Mol. Biol. 283, 255–264.
[54] Bochicchio, B., Pepe, A. and Tamburro, A.M. (2008) Investigating by CD the molecular mechanism of elasticity of elastomeric proteins. Chirality 20, 985–994.
[55] Tamburro, A.M., Guantieri, V., Scopa, A. and Drabble, J.M. (1991) Polypeptide models of elastin: CD and NMR studies on synthetic poly(X-Gly-Gly). Chirality 3, 318–323.
[56] Rauscher, S., Neale, C. and Pomès, R. (2009) Simulated tempering distributed replica sampling, virtual replica exchange, and other generalized-ensemble methods for conformational sampling. J. Chem. Theory Comput. 5, 2640–2662.
[57] Li, N.K., Garcia Quiroz, F., Hall, C.K., Chilkoti, A. and Yingling, Y.G. (2014) Molecular description of the LCST behavior of an elastin-like polypeptide. Biomacromolecules 15, 3522–3530.
[58] Jha, A.K., Colubri, A., Freed, K.F. and Sosnick, T.R. (2005) Statistical coil model of the unfolded state: resolving the reconciliation problem. Proc. Natl. Acad. Sci. U.S.A. 102, 13099–13104.

[59] Toal, S. and Schweitzer-Stenner, R. (2014) Local order in the unfolded state: conformational biases and nearest neighbor interactions. Biomolecules 4, 725–773.
[60] Tanaka, S. and Scheraga, H.A. (1976) Medium- and long-range interaction parameters between amino acids for predicting three-dimensional structures of proteins. Macromolecules 9, 945–950.
[61] Rauscher, S., Baud, S., Miao, M., Keeley, F.W. and Pomès, R. (2006) Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. Structure 14, 1667–1676.
[62] Tompa, P. and Fuxreiter, M. (2008) Fuzzy complexes: polymorphism and structural disorder in protein–protein interactions. Trends Biochem. Sci. 33, 2–8.
[63] Fuxreiter, M. (2012) Fuzziness: linking regulation to protein dynamics. Mol. BioSyst. 8, 168–177.
[64] Sigalov, A., Aivazian, D. and Stern, L. (2004) Homooligomerization of the cytoplasmic domain of the t cell receptor ζ chain and of other proteins containing the immunoreceptor tyrosine-based activation motif. Biochemistry 43, 2049–2061.
[65] Keeley, F.W., Bellingham, C.M. and Woodhouse, K.A. (2002) Elastin as a self-organizing biomaterial: use of recombinantly expressed human elastin polypeptides as a model for investigations of structure and self-assembly of elastin. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357, 185–189.
[66] Bellingham, C.M., Woodhouse, K.A., Robson, P., Rothstein, S.J. and Keeley, F.W. (2001) Self-aggregation characteristics of recombinantly expressed human elastin polypeptides. Biochim. Biophys. Acta – Protein Struct. Mol. Enzymol. 1550, 6–19.
[67] Ross, E.D., Edskes, H.K., Terry, M.J. and Wickner, R.B. (2005) Primary sequence independence for prion formation. Proc. Natl. Acad. Sci. U.S.A. 102, 12825–12830.
[68] Toretsky, J.A. and Wright, P.E. (2014) Assemblages: functional units formed by cellular phase separation. J. Cell Biol. 206, 579–588.
[69] Uversky, V.N., Kuznetsova, I.M., Turoverov, K.K. and Zaslavsky, B. (2015) Intrinsically disordered proteins as crucial constituents of cellular aqueous two phase systems and coacervates. FEBS Lett. 589, 15–22.
[70] Brangwynne, C.P., Eckmann, C.R., Courson, D.S., Rybarska, A., Hoege, C., Gharakhani, J., Jülicher, F. and Hyman, A.A. (2009) Germline P granules are liquid droplets that localize by controlled dissolution/condensation. Science 324, 1729–1732.
[71] Kato, M., Han, T.W., Xie, S., Shi, K., Du, X., Wu, L.C., Mirzaei, H., Goldsmith, E.J., Longgood, J., Pei, J., Grishin, N.V., Frantz, D.E., Schneider, J.W., Chen, S., Li, L., Sawaya, M.R., Eisenberg, D., Tycko, R. and McKnight, S.L. (2012) Cell-free formation of RNA granules: low complexity sequence domains form dynamic fibers within hydrogels. Cell 149, 753–767.
[72] Li, P., Banjade, S., Cheng, H.C., Kim, S., Chen, B., Guo, L., Llaguno, M., Hollingsworth, J.V., King, D.S., Banani, S.F., Russo, P.S., Jiang, Q.X., Nixon, B.T. and Rosen, M.K. (2012) Phase transitions in the assembly of multivalent signalling proteins. Nature 483, 336–340.
[73] Rubinstein, M. and Colby, R.H. (2003) Polymer Physics, Oxford University Press, Oxford.
[74] Huber, M.C., Schreiber, A., von Olshausen, P., Varga, B.R., Kretz, O., Joch, B., Barnert, S., Schubert, R., Eimer, S., Kele, P. and Schiller, S.M. (2014) Designer amphiphilic proteins as building blocks for the intracellular formation of organelle-like compartments. Nat. Mater. 14, 125–132.
[75] Pastuszka, M.K., Okamoto, C.T., Hamm-Alvarez, S.F. and MacKay, J.A. (2014) Flipping the switch on clathrin-mediated endocytosis using thermally responsive protein microdomains. Adv. Funct. Mater. 24, 5340–5347.
[76] MacEwan, S.R. and Chilkoti, A. (2010) Elastin-like polypeptides: biomedical applications of tunable biopolymers. Biopolymers 94, 60–77.
[77] McDaniel, J.R., Callahan, D.J. and Chilkoti, A. (2010) Drug delivery to solid tumors by elastin-like polypeptides. Adv. Drug Deliv. Rev. 62, 1456–1467.
[78] Bhattacharyya, J., Bellucci, J.J., Weitzhandler, I., McDaniel, J.R., Spasojevic, I., Li, X., Lin, C.-C., Chi, J.-T.A. and Chilkoti, A. (2015) A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models. Nat. Commun. 6, 7939.
[79] Dreher, M.R., Liu, W., Michelich, C.R., Dewhirst, M.W. and Chilkoti, A. (2007) Thermal cycling enhances the accumulation of a temperature-sensitive biopolymer in solid tumors. Cancer Res. 67, 4418–4424.
[80] Lampe, K.J., Antaris, A.L. and Heilshorn, S.C. (2013) Design of three-dimensional engineered protein hydrogels for tailored control of neurite growth. Acta Biomater. 9, 5590–5599.
[81] Tatham, A.S. and Shewry, P.R. (2002) Comparative structures and properties of elastic proteins. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357, 229–234.
[82] Cao, Q., Wang, Y. and Bayley, H. (1997) Sequence of abductin, the molluscan "rubber" protein. Curr. Biol. 7, R677–R678.
[83] Li, L., Tong, Z., Jia, X. and Kiick, K.L. (2013) Resilin-like polypeptide hydrogels engineered for versatile biological function. Soft Matter 9, 665–673.
[84] Ardell, D.H. and Andersen, S.O. (2001) Tentative identification of a resilin gene in *Drosophila melanogaster*. Insect Biochem. Mol. Biol. 31, 965–970.
[85] Bochicchio, B., Pepe, A. and Tamburro, A.M. (2001) On (GGLGY) synthetic repeating sequences of lamprin and analogous sequences. Matrix Biol. 20, 243–250.
[86] Green, E.M. and Peter Winlove, C. (2015) The structure and mechanical properties of the proteins of lamprey cartilage. Biopolymers 103, 187–202.
[87] Li, L., Charati, M.B. and Kiick, K.L. (2010) Elastomeric polypeptide-based biomaterials. Polym. Chem. 1, 1160–1170.
[88] Bochicchio, B., Pepe, A. and Tamburro, A.M. (2005) Circular dichroism studies on repeating polypeptide sequences of abductin. Chirality 17, 364–372.
[89] Dutta, N.K., Truong, M.Y., Mayavan, S., Roy Choudhury, N., Elvin, C.M., Kim, M., Knott, R., Nairn, K.M. and Hill, A.J. (2011) A genetically engineered protein responsive to multiple stimuli. Angew. Chem. Int. Ed. 50, 4428–4431.
[90] Su, R.S., Renner, J.N. and Liu, J.C. (2013) Synthesis and characterization of recombinant abductin-based proteins. Biomacromolecules 14, 4301–4308.
[91] Bochicchio, B., Jimenez-Oronoz, F., Pepe, A., Blanco, M., Sandberg, L.B. and Tamburro, A.M. (2005) Synthesis of and structural studies on repeating sequences of abductin. Macromol. Biosci. 5, 502–511.
[92] Li, L., Luo, T. and Kiick, K.L. (2014) Temperature-triggered phase separation of a hydrophilic resilin-like polypeptide. Macromol. Rapid Commun. 36, 90–95.
[93] Lee, J., Macosko, C.W. and Urry, D.W. (2001) Phase transition and elasticity of protein-based hydrogels. J. Biomater. Sci. Polym. Ed. 12, 229–242.
[94] Truong, M.Y., Dutta, N.K., Choudhury, N.R., Kim, M., Elvin, C.M., Nairn, K.M. and Hill, A.J. (2011) The effect of hydration on molecular chain mobility and the viscoelastic behavior of resilin–mimetic protein-based hydrogels. Biomaterials 32, 8462–8473.
[95] Tjin, M.S., Low, P. and Fong, E. (2014) Recombinant elastomeric protein biopolymers: progress and prospects. Polym. J. 46, 444–451.
[96] McGann, C.L., Levenson, E.A. and Kiick, K.L. (2013) Resilin-based hybrid hydrogels for cardiovascular tissue engineering. Macromol. Chem. Phys. 214, 203–213.
[97] Renner, J.N., Cherry, K.M., Su, R.S.C. and Liu, J.C. (2012) Characterization of resilin-based materials for tissue engineering applications. Biomacromolecules 13, 3678–3685.
[98] Stewart, R.J. (2011) Protein-based underwater adhesives and the prospects for their biotechnological production. Appl. Microbiol. Biotechnol. 89, 27–33.
[99] Waite, J.H., Qin, X.X. and Coyne, K.J. (1998) The peculiar collagens of mussel byssus. Matrix Biol. 17, 93–106.
[100] Deming, T.J. (1999) Mussel byssus and biomolecular materials. Curr. Opin. Chem. Biol. 3, 100–105.
[101] Coyne, K.J., Qin, X.X. and Waite, J.H. (1997) Extensible collagen in mussel byssus: a natural block copolymer. Science 277, 1830–1832.
[102] Brazee, S.L. and Carrington, E. (2006) Interspecific comparison of the mechanical properties of mussel byssus. Biol. Bull. 211, 263–274.
[103] Kitamura, M., Kawakami, K., Nakamura, N., Tsumoto, K., Uchiyama, H., Ueda, Y., Kumagai, I. and Nakaya, T. (1999) Expression of a model peptide of a marine mussel adhesive protein in *Escherichia coli* and characterization of its structural and functional properties. J. Polym. Sci. 37, 729–736.
[104] Hwang, D.S. and Waite, J.H. (2012) Three intrinsically unstructured mussel adhesive proteins, mfp-1, mfp-2, and mfp-3: analysis by circular dichroism. Protein Sci. 21, 1689–1695.
[105] Hayashi, C.Y., Shipley, N.H. and Lewis, R.V. (1999) Hypotheses that correlate the sequence, structure, and mechanical properties of spider silk proteins. Int. J. Biol. Macromol. 24, 271–275.
[106] Vepari, C. and Kaplan, D.L. (2007) Silk as a biomaterial. Prog. Polym. Sci. 32, 991–1007.
[107] Heidebrecht, A., Eisoldt, L., Diehl, J., Schmidt, A., Geffers, M., Lang, G. and Scheibel, T. (2015) Biomimetic fibers made of recombinant spidroins with the same toughness as natural spider silk. Adv. Mater. 4, 2189–2194.
[108] Miao, M., Sitarz, E., Bellingham, C.M., Won, E., Muiznieks, L.D. and Keeley, F.W. (2013) Sequence and domain arrangements influence mechanical properties of elastin-like polymeric elastomers. Biopolymers 99, 392–407.